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13. ABSTRACT *(Maximum 200 words)*

Visceral leishmaniasis is an infection of the reticuloendothelial system caused by a protozoan of the *Leishmania donovani* complex. The lack of orally effective agents for visceral leishmaniasis prompted the clinical development of WR6026, a primaquine analog found to be highly active in animal testing. This study was an open-label, dose-escalating trial of WR6026 in the treatment of visceral leishmaniasis caused by *L. chagasi*. The study was performed between October 1996 and July 1998. A Total of 22 volunteer patients were enrolled in a 6 member cohorts. Cohorts 1, 2, 3, 4, and 5 were administered WR6026 capsules at daily doses of 1.0, 1.5, 2.0, 2.5, and 3.25 MKD, respectively, for 28 days. In cohort 1 one patient was withdrawn from the study because diminution of WBC to < 1000/mm3. None of the other 3 patients was cured and the cure rate was 0%. In cohort 2 one patient was cured, and the remaining 5 patients were classified as failures. The cure rate was 1/6=17%. In cohort 3 four patients cured. Two patients, both of whom had varicella during the period of WR 6026 treatment, failed and developed interstitial nephritis. The cure rate was 4/6 = 67%. In cohort 4 three patients showed initial cure, but 2 of these relapsed (cure rate of 1/5=20%). Only 1 patient was entered into cohort 5. Because of renal toxicity his treatment was interrupted on day 21. Because three patients (14%) developed nephropathy the study was terminated.

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T Dietze Feb. 18, 1999

PI - Signature Date

Phase 2 trial of WR6026, an oral 8-Aminoquinoline, in the treatment of Visceral Leishmaniasis Caused by *L chagasi*.

FINAL REPORT
(Aug 1995 - Dec 1998)

TABLE OF CONTENTS

Phase 2 trial of WR6026, an oral 8-Aminoquinoline, in the treatment of Visceral Leishmaniasis Caused by *L chagasi.*

1. Introduction: Visceral leishmaniasis (VL) is a characteristically fatal infection of the reticuloendothelial system (liver, spleen and bone marrow) caused by a protozoan of the *Leishmania donovani* complex. Although more than 90% of the cases can be successfully treated, presently all effective agents are parenteral. The lack of orally effective agents for visceral leishmaniasis prompted the clinical development of WR6026, a primaquine analog found to be highly active in animal testing (Hanson WL 1977, Kinnamon KE, 1978; Chapman WL 1979, Neal RA 1985, Peters W 1980, White MR 1989).

The only Phase 2 study of the efficacy of WR6026 was performed in Kenya (Sherwood, et al, CID 1994). In this study, sixteen patients with VL underwent treatment with WR6026 at doses ranging from 0.75-1.0mg/kg/d for 2 weeks (8 patients) or 1mg/kg/d for four weeks (8 patients). The results included one cure (12%) in the 2-week group and four cures (50%) in the four-week group. The adverse effects included headaches in four patients, and mild abdominal complaints in two patients. Elevations in methemoglobin levels, the main side effect of the drug, were low (2.6% for the 1mg/kg/day x 4 week group). Because neither sufficient efficacy nor significant toxicity was found in the Kenyan study, a second phase II study was undertaken in Brazil. The aim of the study was to dose-escalate with WR6026, given daily for 28 days, until either 90% efficacy or toxicity resulted.

2. Methods: This study was an open-label, dose-escalating trial of WR6026 in the treatment of visceral leishmaniasis caused by *L. chagasi*. The study was performed between October 1996 and July 1998 in the Clinical Research Center of the Unit of Infectious Diseases at the Biomedical Center of the Federal University of Espírito Santo, Vitória, Brazil. Volunteer patients were enrolled in 6 member cohorts. The participants were infected patients from endemic areas for visceral leishmaniasis in the states of Espírito Santo, Bahia and Minas Gerais.

Subjects enrolled were of both genders, with ages ranging from 6 to 50 years. The inclusion criteria for all subjects were: **a)** a clinical diagnosis of visceral leishmaniasis with symptomatic disease, **b)** parasitological demonstration of Leishmania: visualization of Leishmania amastigotes on Giemsa Diff-Quik stained splenic aspirates or positive culture in diphasic blood agar medium with an overlay of 0.1 ml Schneider's Drosophila medium, (Gibco, Grand Island , NY), supplemented with 20% heat-inactivated fetal calf serum and 100 ug/ ml of gentamicin (Grögl et al., Exp Parasit 1989).

The exclusion criteria included clinical contraindication to splenic aspirate, any history of prior anti-Leishmania therapy, evidence of serious underlying disease (cardiac, renal, hepatic, or pulmonary) including serious infection other than visceral leishmaniasis, immunodeficiency or antibody to HIV, severe protein and/or caloric malnutrition (Kwashiorkor, Marasmus), G6PD deficiency, pregnancy, hemoglobin concentration less than 5g/100 ml, WBC fewer than 1000, platelets fewer than 30,000/mm^3, and a significant (>3x control values) deviation in serum chemistries (blood urea nitrogen, creatinine, ALT, AST).

Informed consent was obtained from all patients or parents of minors. This study was approved by the institutional review board at the Federal University of Espírito Santo.

2.1 Drug Administration: Cohorts 1, 2, 3, 4, and 5 were administered WR6026 capsules (WRAIR Chemical Inventory) at daily doses of 1.0 MKD, 1.5 MKD, 2.0 MKD, 2.5 MKD, and 3.25 MKD, respectively, for 28 days with water 1 hr before breakfast and under supervision.

2.2 Determination of Efficacy: Patients were classified according to the following mutually exclusive categories:

a) **Initial cure:** no parasite seen by smear or culture of splenic aspirate or if the spleen is too small for aspiration, bone marrow aspiration.

b) **Initial improvement:** at least 2 log decrease in parasites on smear, and clinical improvement defined by at least one of the following changes: patient became afebrile, liver and/or spleen size regressed by 50% of pre-treatment

values, body weight increases by 1 kg/week, increased in hemoglobin by 0.5g/dl/week, white blood count increased 500/μl^3/week. Patients with initial improvement were followed with further organ aspiration to determine if parasites completely remitted, and with further clinical examinations for 12 months.

c) **Final cure:** no parasites seen in organ aspirates and no evidence of infection by the end of 12 months of follow-up.

d) **Failure:** lack of initial cure or initial improvement, relapse after initial cure or initial improvement, lack of progression of initial improvement to final cure.

2.3 Determination of drug toxicity: Each day patients were questioned about symptoms suggesting possible drug side effects, including nausea, abdominal discomfort, and headache. In addition vital signs were recorded and examined. Laboratory tests other than G6PD were repeated weekly during the 4-week treatment period and during follow up periods.

2.4 Drug concentrations: Plasma was drawn weekly just prior to drug dosing for determination of trough levels of WR6026 and its n-desethyl metabolite WR211789. Plasma was analyzed by HPLC.

3. Results

3.1 Patient characteristics: The enrolled patients were primarily young adults who presented with the characteristic picture of moderate kala-azar: splenomegaly of approximately 8 cm below the left costal margin, moderate decreases in the formed elements of the blood, and parasitemia of approximately 3.5 log units (Table 1).

Table 1. Pre-treatment demographics characteristics of patients.

Parameters	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
Dose (mg/kg/day)	1.0 (n = 4)	1.5 (n = 6)	2.0 (n=6)	2.5 (n=5)	3.25 (n=1)
Male/female ratio	4/0	5/1	5/1	3/2	1/0
Age (y)	19±2.5 (16-22)	32.8±12.9 (11-49)	23.8±12.4 (9-39)	23.8±8.8 (10-31)	22
WR6026 daily dose (mg/kg)	1	1,5	2	2,5	3,25
Duration of disease (months)	4.7±2.9 (1-8)	3.5±1.2 (1-4)	6.2±4.2 (2-12)	6.6±9.8 (1-24)	1
Amastigotes (splenic aspiration)	3±1.2	3.5±0.8	2.8±0.75	4±1.2	3
Physical examination					
Axillary temperature (^{0}C)	38.5±1.2 (37-39.7)	38.6±1.3 (37.2-40.5)	38.5±1.3 (36.4-40.3)	38.5±0.5 (37.8-39)	38.7
Spleen size (cm)	8.2±3.3 (5-12)	9.2±3.2 (5-13)	10.5±3.9 (6-14)	6.9±2.7 (3-10)	5
Hematologic and serum chemistry					
Methemoglobin	0.46±0.18 (0.3-0.7)	0.7±0.37 (0.3-1.2)	0.7±0.1 (0.5-0.9)	0.5±0.18 (0.3-0.7)	0.2
Hemoglobin g/dl (normal 12-17)	9.2±0.93 (7.8-9.8)	7.7±1.5(6.5-10.1)	8.7±0.9(7.3-10.2)	8.4±1.6 (7.1-11)	8.8
WBC 1.000/mm^3 (normal 5-10)	2.9±0.76 (2-3.7)	2.3±0.89 (1.1-3.3)	2.2±0.91 (1.2-3.6)	2.8±1 (1.7-3.9)	2.800
Platelet 1.000mm^3 (normal 200-400)	157±58 (99-210)	95±39 (32-138)	100±32 (55-127)	142±45 (92-151)	111
Albumin g/dl (normal 3.5-5.5)	2.8±0.52 (2.3-3.3)	2.9±0.81 (1.9-4.1)	2.7±0.5 (2.3-3.7)	2.7±1.1 (0.9-3.8)	2.9
Gamaglobulin mg/dl (normal 0.5-1.6)	3.04±1.2 (1.76-4.8)	2.19±0.8 (1.26-3.7)	4±1.5 (1.4-5.7)	3.7±1.4 (2.03-5.9)	3.2
BUN mg/100mL (normal 10-20)	13.2±1.7 (11-15)	13.8±3.6 (10-19)	12.8±2.7 (9-16)	9±3.4 (6-14)	12
Creatinine mg/100mL (normal 0.6-1.2)	0.7±0.2 (0.5-1)	0.7±0.14 (0.5-0.9)	0.8±0.3 (0.4-1.3)	0.7±0.1 (0.6-1)	1
SGOT U/L (normal 4-32)	34±10.5 (21-43)	34.6±22.6 (16-74)	53.8±31.8 (8-106)	33±18.3 (14-68)	86

Data represent the mean±SD(range) with exception of cohort 5 because there was only one patient.

Table 2. Results of Therapy

Parameters	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
Dose (mg/kg/day)	1.0	1.5	2.0	2.5	3.25
	(n = 4)	(n = 6)	(n=6)	(n=5)	(n=1)
Outcome					
Initial Presumed Cure	1	1	5	3	1
Partial Clinical Response	0	0	0	0	NA
Final outcome					
Clinical Cure (%)	0 (0%)	1 (17%)	4 (67%)	1 (20%)	0 (0%)
Blood Levels of Drug					
WR6026 day 7	37±23	302±341	199±105	526±716	ND
WR6026 day 28	21±12	145±253	114±90	414±614	ND
WR211789 day 7	39±22	132±116	97±32	197±105	ND
WR211789 day 28	33±23	82±72	69±41	212±211	ND
Physical examination at day 28					
Axillary temperature (^{0}C)	37.5±0.3 (37.2-38)	37.3±1.1 (36-39.4)	37.4±1 (36.4-39.5)	36.4±0.2 (36.1-36.7)	38.7
Spleen size (cm)	5.4±2.8 (3-8.5)	7.1±5.1 (0-13)	4.9±6 (0-14)	3.7±3.1 (0-7)	5
Hematologic and serum chemistry at day 28					
Methemoglobin day 7	3.3±4.2 (0.7-9.6)	3.1±1.5 (1.4-5.4)	4.3±4.5 (1.2-13.3)	5.4±4.1 (1.1-7.2)	3.5
Methemoglobin day 28	2.6±3.1 (0.7-7.2)	4.1±2.3 (1.6-6.9)	5.4±2.6 (2.8-9.1)	5.7±2.3 (2.1-8.3)	5.6
Hemoglobin g/dl (normal 12-17) – day 28	9.45±1.5 (8-11.2)	9.3±1.1 (7.1-10.3)	10.2±1.7 (8.8-13.3)	10.2±2.1 (7.6-12.5)	10.2
WBC 1.000/mm^3 (normal 5-10) – day 28	2.3±1.3 (1-5.5)	3.8±2.6 (1.3-8.2)	3.1±1.2 (2-4.8)	3.5±1.4 (1.7-5.3)	4880
Platelet 1.000mm^3 (normal 200-400) – day 28	198±33 (150-228)	187±115 (86-406)	149±66 (100-126)	191±64 (116-292)	252
Albumin g/dl (normal 3.5-5.5) – day 28	3.5±0.2 (3.3-3.7)	4.1±0.9 (3.2-5.4)	3.9±0.6 (3.1-5)	3.5±1.3 (2-4.8)	2.2
Gamaglobulin mg/dl (normal 0.5-1.6)	3.2±0.9 (2.3-4.5)	2.3±1.3 (1.1-4)	4.3±1.7 (1.4-6.2)	3.5±2.7 (1.3-8.1)	1.6
BUN mg/100mL (normal 10-20)	10±0	12.3±2.2 (10-15)	10±2.1 (9-13)	10.6±2.8 (7-15)	19
Creatinine mg/100mL (normal 0.6-1.2)	0.7±0.1 (0.7-0.9)	1.01±0.1 (0.8-1.2)	0.9±0.4 (0.5-1.6)	0.8±0.2 (0.5-0.9)	1.6
SGOT U/L (normal 4-32)	28.5±9.4 (17-40)	33.3±14.8 (15-50)	40.4±28.4 (20-89)	35.8±14.6 (21-59)	41

Data represent the mean±SD (range) with exception of cohort 5 because n=1.

3.2 Efficacy: Table 2 summarizes the results of treatment with WR6026 in the five cohorts. In cohort 1 (1 mg/kg/day) one patient showed diminution of WBC to < 1000/mm3 which was ascribed to advancing disease. None of the other 3 patients was cured and the cure rate was 0%. In cohort 2 (1.5 mg/kg/day) one patient was cured, and the remaining 5 patients were initially improved, but showed no further improvement in disease parameters upon follow up and were ultimately classified as failures. The cure rate was 1/6=17%. In cohort 3 (2.0 mg/kg/day) 4 patients cured and did not relapse. Two patients, both of whom had varicella during the period of WR 6026 treatment, failed. The cure rate was 4/6 = 67%. In cohort 4 (2.5 mg/kg/day) 3 patients showed initial cure, but 2 of these relapsed with a final cure rate of 1/5=20%. Only 1 patient was entered into cohort 5 (3.25 mg/kg day). Because of renal toxicity his treatment was interrupted on day 21. Although he was classified as initial cure, he relapsed after 10 months of therapy.

3.3 Toxicity: Clinical side effects frequently ascribed to drugs (gastrointestinal, headache) were found in < 5% of patients in this open-label trial. Laboratory abnormalities seen in WR6026 animal studies were methemoglobinemia, which prevented dosing above 3 mg/kg/day in dogs, and liver enzyme elevations. In this study, blood methemoglobin levels reached approximate steady state at 7 days. We were surprised that mean methemoglobinemia was <6% at a dose of approximately 3 mg/kg day, and that there was no statistically significant between peak blood methemoglobin levels and daily dose (simple regression p=0.36, R2= 0.05) Liver enzymes such as AST and triglycerides also did not rise (Table 2).

An unexpected side effect was nephropathy in 3 patients. Two patients, both in cohort 4, neither of whom had pre-existing antibodies to varicella, manifested primary varicella infection. The first varicella patient demonstrated vesicles 4 days after starting WR6026 therapy. Forty-four days later (20 days after WR 6026 had finished) his creatinine was 1.8mg/dl (normal 0.6-1.2mg/dl) up from 0.9mg/dl from at the commencement of the study. Renal biopsy was performed and steroids were started. The biopsy revealed "interstitial nephritis and areas of acute tubular necrosis in regeneration".

The second varicella patient developed cutaneous vesicles 2 days after finishing WR6026. Fourteen days after finishing WR6026, his creatinine was 3.3mg/dl increased from 1.3mg/dl at the commencement of the study. Forty-one days after finishing WR6026, the creatinine reached 5.6mg/dl. The patient underwent kidney biopsy which again revealed "interstitial nephritis and areas of acute tubular necrosis in regeneration". Immunohistochemical examination of the biopsy was negative for Leishmania, adenovirus, herpes simplex I and II, and cytomegalovirus. The biopsy was also negative for varicella DNA via *in situ* hybridization and PCR. Kidney function eventually recovered with steroid therapy.

Because both patients with nephropathy also had primary varicella, it was thought that the combination of kala-azar, WR6026 , and varicella was needed to produce this side effect. Therefore, one patient in cohort 5 (3.25 mg/kg/day) was entered. When this patient demonstrated a rise in creatinine (2mg/dl) on day 24 with a creatinine clearance of 29ml/min, administration of WR6026 to this patient was stopped and the study was terminated. Kidney function returned to normal 18 days after the drug was stopped.

3.4 WR6026 blood levels: Plasma levels of WR 6026 and a major metabolite were in the majority of cases higher on day 7 than on day 28. Although week 1 trough levels showed an absolute increase in cohort 1 and cohort 2 (table 2), there was no correlation between dose and drug levels (simple regression: p=013, R2=0.12).

4. Discussion

The need for an oral anti-leishmanial drug and encouraging data in the previous Kenyan trial prompted this study of WR6026 against Brazilian kala-azar. The current study yielded a number of unanticipated results with respect to both efficacy and toxicity. Given that the cure rate in the Kenyan study with a dose of 1MKD at 28 days was 50%, drug efficacy in the current study was less than expected. The starting dose of 1MKD cured none of 4 patients. In addition efficacy did not uniformly increase with escalating doses as best demonstrated by comparing the cure rates of Cohorts 3 and 4 (67% vs, 20%).

The side effect profile in the current study also deviated from those reported in previous studies. In earlier experimental studies in dogs and in clinical trials with HIV patients, dose escalation yielded corresponding increases in methemoglobinemia, a result of oxidant action of the drug on hemoglobin, which ultimately became dose-limiting toxicity. A mean of 30% methemoglobin blood levels peaking at 4 weeks was seen in dogs administered 3 MKD for 13 weeks. Two of 6 HIV patients administered approximately 2 MKD for 3 weeks had methemoglobinemia values in excess of 20%. In the current study despite a slightly higher peak dose (3 MKD) and long duration of treatment (4 weeks), maximal mean methemoglobin blood levels remained below 6%. Clinically significant renal toxicity was a side effect not reported in the dog and HIV patient studies. Two of the 3 patients developing nephropathy in the current study suffered from coincidental varicella infection. While varicella in some cases has been associated with glomerulonephritis, the damage reported in the renal biopsies from the current study affected the tubules and the interstitium. While nephrotoxicity is not a well documented side effect with drugs of the primaquine family, it is possible that dose/time dependant drug toxicity was responsible for the interstitial nephritis. If this were the case, one might expect more cases of this side effect with higher doses.

The pharmacokinetics of WR6026 are poorly understood and the unanticipated results in our study could be explained by unusual metabolic phenomena. In the dog, radioactive WR6026 is virtually 100% orally bioavailable, but only 4% of the orally administered drug can be recovered as WR6026 due to presumed first-pass metabolism (Hawkins et al. 1989). After both intravenous and oral administration, approximately 6% of radiolabelled WR6026 are excreted into dog feces and 29% into dog urine. In clinical phase 1 study, 14% of orally administered WR6026 was excreted into the urine, but only 7% of this was identified as WR6026 and about 3% was identified as the metabolite WR211789. Thus, WR6026 and WR211789 together represent about 10% urinary 8-aminoquinoline species and, if urinary excretion represents bodily drug, about 10% of drug in the body (Theoharides et al. 1987).. The vast majority species formed from WR6026 and present in the body are therefore unknown and their efficacy *versus* Leishmania and toxicity to mammalian cells are equally unknown. It can be

hypothesized that marked differences in metabolism, and therefore in efficacy and toxicity could occur in different hosts, in different disease states, and (due to auto-oxidation) with different doses.

The future of WR6026 as an anti-leishmanial agent depends on the demonstration of the efficacy without undue toxicity in further trials. If *Leishmania* species other than *L. chagasi* are more sensitive to WR6026, or patients from different regions metabolize drug differently, or higher number of patients are examined the relatively discouraging results from this trial might contradicted. The importance of having an oral agent for visceral leishmaniasis makes such further investigation worthwhile.

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